MOLECULAR ONCOLOGY 9 (2015) 44–57

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Infiltrating T cells promote prostate cancer metastasis *via* modulation of FGF11→miRNA-541→androgen receptor (AR)→MMP9 signaling

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ARTICLE INFO

Article history:
Received 28 June 2014
Received in revised form
14 July 2014
Accepted 14 July 2014
Available online 29 July 2014

Keywords:
Prostate cancer
T cells
Androgen receptor
Tumor metastasis
Tumor microenvironment

ABSTRACT

Early clinical studies suggested infiltrating T cells might be associated with poor outcomes in prostate cancer (PCa) patients. The detailed mechanisms how T cells contribute to PCa progression, however, remained unclear. Here, we found PCa cells have a better capacity to recruit more CD4(+) T cells than the surrounding normal prostate cells *via* secreting more chemokines-CXCL9. The consequences of more recruited CD4(+) T cells to PCa might then lead to enhance PCa cell invasion. Mechanism dissection revealed that infiltrating CD4(+) T cells might function through the modulation of FGF11→miRNA-541 signals to suppress PCa androgen receptor (AR) signals. The suppressed AR signals might then alter the MMP9 signals to promote the PCa cell invasion. Importantly, suppressed AR signals *via* AR-siRNA or anti-androgen Enzalutamide in PCa cells also enhanced the recruitment of T cells and the consequences of this positive feed back regulation could then enhance the PCa cell invasion. Targeting these newly identified signals *via* FGF11-siRNA, miRNA-541 inhibitor or MMP9 inhibitor all led to partially reverse the enhanced PCa cell invasion. Results from *in vivo* mouse models also confirmed the *in vitro* cell lines in co-culture studies. Together, these results concluded that infiltrating CD4(+) T cells could promote PCa metastasis *via* modulation of FGF11→miRNA-541→AR→MMP9 signaling. Targeting these newly identified signals may provide us a new potential therapeutic approach to better battle PCa metastasis.

1. Introduction

The incidence of prostate cancer (PCa) across the Western World is increasing at an alarming rate and becoming the most common form of cancer in men in the USA and the second leading cause of cancer death (Jemal et al., 2008). Many factors, including age, family history, and diet may influence the PCa development, however, the detailed mechanism(s) remain unclear (Maitland and Collins, 2008).

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http://dx.doi.org/10.1016/j.molonc.2014.07.013
1574-7891/

The androgen receptor (AR) has been recognized as the key player in the PCa progression (Chang et al., 2013, 1988; Heinlein and Chang, 2004; Wen et al., 2013). Importantly, more and more data suggested that AR might have duel roles to either promote PCa proliferation or suppress PCa metastasis (Lai et al., 2012; Lin et al., 2013a, 2013b; Niu et al., 2008a, 2008b, 2010). Using knocked-out AR in selective cells, Niu et al. (Niu et al., 2008a, 2008b, 2010) and Lai et al. (Lai et al., 2012) concluded that PCa stromal AR could promote PCa progression and AR in epithelial luminal cells could also play positive roles to maintain PCa survival. However, AR in epithelial basal intermediate cells might play a suppressor role in PCa metastasis (Niu et al., 2008a, 2008b, 2010). Other reports suggested that AR might function as suppressor in PCa metastasis *via* modulation of the epithelial–mesenchymal transition (EMT) and stem cell population (Sun et al., 2012; Tu and Lin, 2012).

The prostate tumor microenvironment (pTME) with many inflammatory immune cells may play important roles during PCa development and progression (Fang et al., 2013; Izumi et al., 2013; Lin et al., 2013a; Omabe and Ezeani, 2011; Reebye et al., 2011; Vendramini-Costa and Carvalho, 2012; Wang et al., 2013; Yates, 2011). Among these infiltrating immune cells, T cells with CD4(+) and/or CD8(+) were found to be associated with PCa (Steiner et al., 2002) and early clinical studies suggested infiltrated CD4(+) T cells might be linked to the poor outcome in patients with PCa (McArdle et al., 2004). The detailed mechanism how these CD4(+) T cells were recruited to the PCa and how they contributed to the PCa metastasis, however, remained unclear.

Here we demonstrated that infiltrated CD4(+) T cells could enhance PCa cell invasion and mechanism dissection found the infiltrated CD4(+) T cells might go through modulation of FGF11→miRNA-541→AR→MMP9 signaling to influence the PCa metastasis.

2. Materials and methods

2.1. Patients

We found 20 patients whose biopsies showed clinical evidence of PCa by biopsy. All these patients were treated with radical prostatectomy and no other therapy before surgery. Each patient biopsy tissue was divided into 2 groups: one was the PCa area and the other was the adjacent normal tissue area. The detailed information of patients was shown in Table 1.

Table 1 – The clinical information from 20 PCa patients.

Patient number (*n*)	20
Age (years)	63.8 ± 5.6
*tPSA (ng/ml)	21.57 ± 19.63
**f/tPSA	0.12 ± 0.05
TNM2002 AJCC*	
T1	15
T2	5
pT2a	3
pT2b	2
Gleason score	
Gleason 6	17
Gleason 7	1
Gleason 8	2

*tPSA: total prostate-specific antigen.
**f/tPSA: the ratio between free PSA and total PSA.

2.2. Cell culture

The LNCaP and CWR22RV1 cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA), the C4-2 cell line was a gift from Dr. Jer-Tsong Hsieh (Southwestern Medical Center, USA) and grown in RPMI-1640 media (Invitrogen #A10491, Grand Island, NY, USA) containing 1% penicillin and streptomycin, supplemented with 10% fetal bovine serum (FBS). The immortalized non-transformed RWPE-1 prostate epithelial cell line was purchased from American Type Culture Collection and grown in keratinocyte serum free medium (K-SFM) supplemented with bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). CD4(+) T-lymphocytic cell lines HH and Molt-3 were acquired from the American Type Culture Collection (ATCC# CRL-2105 and CRL-1552 respectively), and maintained in 10% heat-inactivated fetal bovine serum RPMI media with 1% Pen/Strep. All cell lines were cultured in a 5% CO_2 humidified incubator at 37 °C.

2.3. Reagents

Monoclonal anti-CXCL9 antibodies were purchased from R&D systems (Minneapolis, MN, USA), and 500 μg/ml stock was reconstituted in phosphate buffered saline (PBS). For anti-CXCL9 treatment, stocks were adjusted to a final concentration of 6 μg/ml. MMP9 inhibitor was purchased from ABGene (Pittsburgh PA, USA) and was adjusted to a final concentration of 5 μg/ml. For cell treatment, Enzalutamide were adjusted to a final concentration of 10 μM.

2.4. Migration assay

The PCa cells and normal prostate RWPE-1 cells at 1×10^5 were plated into the lower chamber of the transwells with 5 μm pore polycarbonate membrane inserts (Corning, #3422, Corning, NY, USA). 1×10^5 HH or Molt-3 cells were plated onto the upper chamber. After 6 h, the cells migrated into the lower chamber media were collected and counted by the Bio-Rad TC10 automatic cell counter. Each sample was assayed in triplicate and the experiments were repeated twice.

2.5. Invasion assay

For *in vitro* invasion assays, the upper chambers of the transwells (Corning; 8 μm pores) were pre-coated with diluted growth factor-reduced Matrigel (1:4 serum free RPMI media) (BD Biosciences, Sparks, MD). Before invasion assays, PCa cells were co-cultured with HH or Molt-3 for 48 h in 6-well transwell plates (Corning; 0.4 μm). 1×10^5 of HH or Molt-3 cells were plated onto the upper chamber and 1×10^6 PCa cells were plated into the lower chamber. The conditioned media (CM) or control media were collected, diluted with 10% FBS RPMI